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13. SUPPLEMENTARY NOTES

14. ABSTRACT
The purpose of the project is test the hypothesis that interventions that reduce the function of a sodium ion channel, Nav1.8, that is selectively expressed in primary afferent neurons (especially nociceptors) ameliorate reflex hypersensitivity and pathological pain-related motivational/cognitive alterations caused by traumatic spinal cord injury (SCI). The first phase of the project was accomplished, with a major paper published describing how effective antisense knockdown of Nav1.8 eliminates SCI-induced spontaneous activity in nociceptors, reverses mechanical and heat hypersensitivity of hindlimb withdrawal reflexes, and ameliorates ongoing, spontaneous pain. During the past year we have investigated effects of a selective Nav1.8 antagonist, A-803467, on heat hypersensitivity, mechanical hypersensitivity, spontaneous pain, and anxiety. We have also found that blockade of the cAMP-PKA signaling pathway, known to enhance Nav1.8 activity, eliminates SCI-induced spontaneous activity. These studies will be completed during the 6-month extension without funds. During the past two quarters we found evidence that an inexpensive but nonspecific Nav1.8 inhibitor, ambroxol, does not reduce pain-associated behavior, while the more specific antagonist, A-803467, continues to show promise not only for reducing hyperreflexia, spontaneous pain, and evoked pain, but also anxiety after SCI.

15. SUBJECT TERMS
Spinal cord injury, chronic pain, spontaneous pain, evoked pain, anxiety, primary nociceptors, Nav1.8, hyperreflexia

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1. INTRODUCTION

The purpose of this project is to test a novel approach to treating chronic pain and other complications of spinal cord injury (SCI) using a preclinical rat model. Over 40,000 veterans have SCI, as well as many active members of the armed services, and a majority of these people endure intractable pain and potentially related chronic problems such as anxiety and gastrointestinal dysfunction for the rest of their lives. Most investigators have assumed that the critical mechanisms driving SCI pain are located within the central nervous system (CNS) and involve direct effects of the injury and/or associated neuroinflammation on pain pathways (Finnerup and Baastrup, 2012; Walters, 2012; Walters, 2014)). Early evidence that primary sensory neurons, and especially primary nociceptors, are involved in neuropathic SCI pain came from observations of enhanced nociceptor growth after SCI (Bedi et al., 2012). Primary nociceptors are the first neurons within pain pathways and thus their electrical activity leads to the conscious sensation of pain as well associated reflex responses. These sensory neurons are specialized for the detection of bodily injury and inflammation and are normally electrically silent, firing action potentials only when their peripheral branches are activated by stimuli that can produce pain (fortunately, an infrequent occurrence for most people). This award enables rigorous tests of our hypothesis that prominent aspects of chronic pain and hypersensitivity caused by SCI can be ameliorated effectively by interventions that selectively block spontaneous electrical activity in primary nociceptors. Four years ago we reported (Bedi et al., 2010) the unexpected discovery that primary nociceptors in rats that have received a contusive spinal injury (controlled experimental bruising of the spinal cord) months earlier continuously fire action potentials without any extrinsic stimulation ("spontaneous activity," SA), even when the recorded nociceptor is removed from the body and isolated from all other cells. Electrical activity in any nociceptor would be expected to excite pain pathways and thereby promote pain sensations, and so it was not surprising to find that this chronic nociceptor SA was closely correlated with behavioral measures of pain; animals exhibiting pain showed a high incidence of nociceptor SA whereas apparently pain-free animals did not. More direct evidence that activity in primary nociceptors helps to maintain SCI pain came from our finding that antisense knockdown of TRPV1 channels or pharmacological blockade of TRPV1 channels -- which are expressed most abundantly in nociceptors -- reduced SA after SCI and caused a dramatic reversal of reflex hypersensitivity (Wu et al., 2013). Importantly, the nociceptors exhibiting SA after SCI possess an ion channel, Nav1.8, that in the nervous system is only expressed by primary sensory neurons, and primarily in nociceptors (Shields et al., 2012). We found that a drug that selectively blocks Nav1.8 channels blocks SA in nociceptors after SCI. These recent discoveries led directly to the hypothesis and associated experiments in this project. We are testing the prediction that interventions that reduce Nav1.8 function -- specifically antisense knockdown of Nav1.8 expression and inhibition of Nav1.8 channels using two different drugs -- will reduce chronic pain and other debilitating behavioral effects (as well as SA and hyperexcitability in nociceptors) after SCI. To model chronic dysfunction after SCI, animals are tested 6 to 12 weeks after injury. A novel and potentially important part of our experimental design has been to apply operant measures of ongoing, spontaneous pain and other emotional effects after SCI. These provide more relevant models of pain and suffering after SCI than the hyperreflexia measures that almost all previous studies of SCI pain have depended on. Our experiments thus far have confirmed some of our major predictions, but have also revealed unexpected behavioral consequences of SCI that complicate the measurement of pain and hyperreflexia, while suggesting an even more profound than anticipated role for nociceptor SA in persistent suffering produced by SCI.

2. KEYWORDS

spinal cord injury, chronic pain, spontaneous pain, evoked pain, anxiety, primary nociceptor, spontaneous activity, Nav1.8, hyperreflexia

3. OVERALL PROJECT SUMMARY

Major objectives (aims) of the project

Aim 1 (Tasks 1b, 1c): Test the hypothesis that chronic reflex hypersensitivity in a rat contusive SCI model is reduced by blocking spontaneous activity (SA) in nociceptors via reduction in Nav1.8 activity achieved by either knocking down Nav1.8 channel expression or by applying a highly specific Nav1.8 blocker, A-803467. 95% completed.

Aim 2 (Tasks 1d, 1e): Test the same hypothesis by seeing if a less specific but much less costly Nav1.8 blocker, ambroxol, can be used for both brief and prolonged attenuation of behavioral hypersensitivity after SCI (Tasks 1d, 1e). 100% completed.

Aim 3 (Task 1f): Test the prediction that chronic visceral hypersensitivity after SCI can be reduced by decreasing the activity of Nav1.8 channels. This task was begun with Dr. Hongzhen Hu, a collaborator in the department with expertise in visceral pain testing, but had to be abandoned when Dr. Hu left the institution and because it was found that repeated testing of the same animals with multiple types of pain testing caused excessive stress.

Aim 4 (Tasks 2a-2e): Show that decreasing Nav1.8 activity or expression reduces evoked and spontaneous motivational and cognitive features of pain-related behavior after SCI. 80% completed.

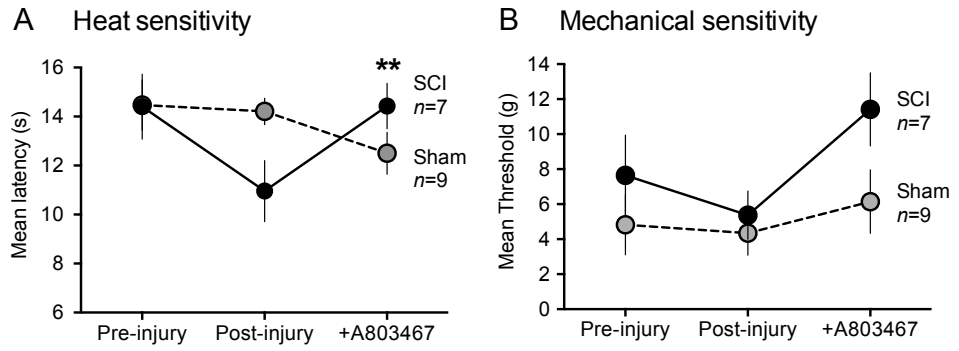
Accomplishments under each task

Task 1a - Institutional and DOD animal use approvals. Accomplished.

Task 1b – Investigate reflex hypersensitivity effects of knocking down Nav1.8 expression. In August, 2014 we published a paper (Yang et al., 2014) documenting our findings from work completed in Year 2 of this award on the suppression of SCI-induced hyperreflexia by Nav1.8 knockdown.

Task 1c – Investigate reflex hypersensitivity effects of selectively blocking Nav1.8 activity with low- or high doses of A-803467.

Fig.1

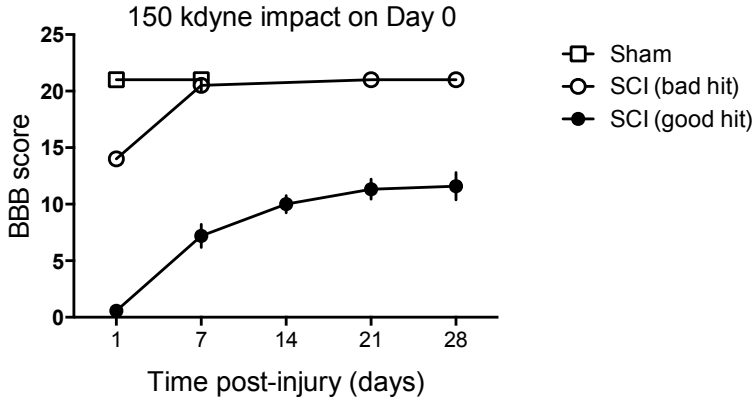


In Year 3 we have continued to test whether delivery of had been the most selective Nav1.8 antagonist available, A-803467, reduces SCI-induced hyperreflexia. Because of low bioavailability, this drug is very expensive to use for whole-animal studies; even after a discount of nearly 70% that we negotiated with Selleckchem for volume purchases, it costs about $60 per i.p. injection per rat to test behavioral effects of A-803467. Consequently, we limited this Aim to testing the acute effects of a single injection of the drug, even though prolonged or repeated application would be expected to produce effects closer to that produced by antisense knockdown. At the end of Year 2 we found that a relatively low dose, 30 mg/kg (Jarvis et al., 2007), had very weak effects, if any, so we focused our studies on the higher dose, 100 mg/kg. The complex results obtained are summarized in Fig.1. The effect on hypersensitivity to radiant heat stimulation of the hindpaws seemed clear (Fig.1A). A-803467 injection 20 min before testing caused a significant reversal of the withdrawal latency that had been reduced by SCI when tested ~1 week earlier (more than 1 month after injury). This result suggested that SCI-induced hypersensitivity to heat

stimulation has a moment-to-moment dependence upon electrical activity requiring Nav1.8 channels, and thus encouraged further investigation into the potential use of Nav1.8 antagonists for the treatment of SCI pain. However, the effects of A-803467 on mechanical sensitivity in the same animals (Fig.1B) were more difficult to interpret, and led us to consider the possibility that the drug effects were variable because the degree of pain produced by our SCI protocol at the time had become more variable. Thus, in Year 3 we have focused on improving our surgical and testing methods.

Surgical and testing procedures were optimized by sending Max Odem and Dr. Alexis Bavencoffe to the Spinal Cord Injury Research Training Program at Ohio State University (OSU) in May of 2015. This intense, 2-week course delayed our planned research schedule but the benefits have been extremely valuable. Max returned to practice SCI surgeries under the guidance of Dr. Qing Yang (who had to leave the project), and Dr. Juan Herrera, who is an independent SCI investigator within this institution. On the basis of information from the Ohio State course and input from Drs. Yang, Herrera, and veterinarians from our animal facility, we sought to improve the effectiveness of our SCI procedures and to reduce mortality and morbidity caused by sub-optimal design and implementation of these procedures. Our major accomplishment during this quarter was to substantially improve our surgical procedures. This advance is illustrated in the BBB scores for hindlimb motor function in the animals that Max performed spinal contusion surgery on (Fig.2). In contrast to our relatively high attrition rates during the past two years, none of the 10 animals given SCI by Max died or developed infections, and only 2 had to be discarded because of “bad hits” on the spinal cord (glancing blows).

Fig.2



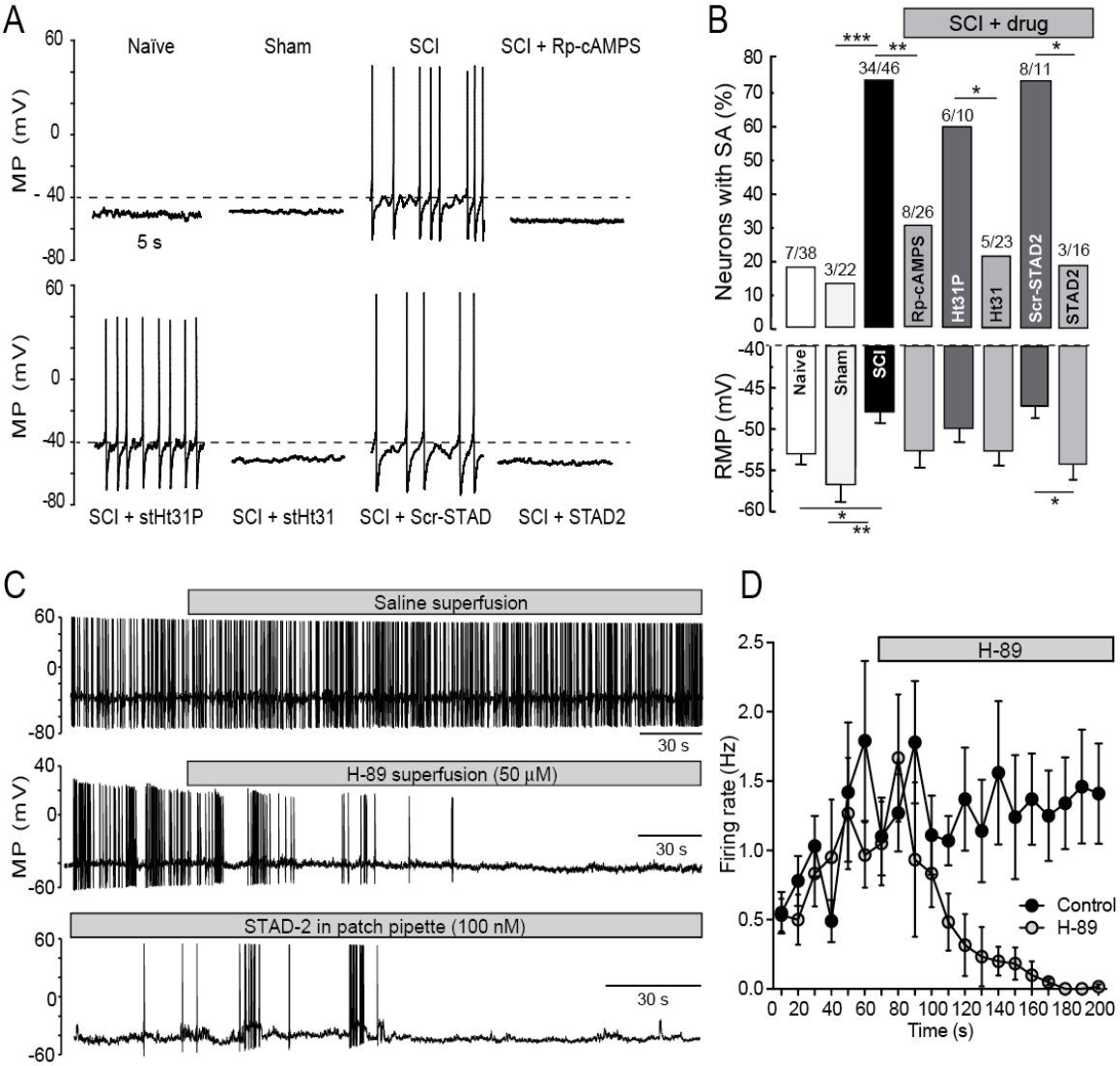
This advance reflects improved sterile procedures and a more sophisticated understanding of how to control the Infinite Horizon device. At the OSU course, Max and Alexis learned that our contusion procedures diverge somewhat from those practiced by the leading rodent SCI research labs at OSU. Some of these differences, such as whether blood accumulating over the exposed spinal cord is absorbed with sterile Q-tips during the procedure, may influence how much chronic pain is induced. Third, Dr. Philip Popovich (one of the leaders in the rodent SCI behavior field) informed them of unpublished observations showing that a higher impact contusive force (200 kdynes vs the 150 kdyne force we currently use) produces more reliable pain behavior in SCI rats.
The stage is now set for more efficient testing of the effects on SCI pain of the two Nav1.8 antagonists to be investigated in the final quarters of this project (during the 6-month extension without funds [EWOF] period).

During Year 2 Ms. Julia Hadden was replaced by Mr. Max Odem as the animal tester. Any time a new tester joins a project there is the possibility that subtle differences in testing techniques will add to response variability. This is more likely in the von Frey filament tests than the radiant heat tests because of greater degrees of freedom in hand delivery of the filament when trying to maintain consistent timing and velocity to a precise spot on the paw. Importantly, we also concluded that with our procedures, the male tester inadvertently caused more stress to the rats than did the female testers. Reinforcing this conclusion was publication of an important paper documenting a remarkably strong stress-induced analgesia in rats and mice produced by olfactory cues from male experimenters (or even their clothing) several feet away from the rodents (Sorge et al., 2014). Although naive animals habituate to this effect with sufficient exposure to a familiar male, and Mr. Odem now spends extensive time familiarizing himself with the rats before he conducts any tests, the possibility remains that SCI animals are extra sensitive to potential threats and may not completely habituate to a male tester. We recently hired a female tester (paid by the PI’s endowment funds) and she will conduct most of the remaining behavioral tests under Mr. Odem’s supervision during the EWOF period.

We have also examined other antagonists that are likely to reduce Nav1.8 function, albeit indirectly. These studies made use of animals that had been used for the behavioral pharmacological studies in the

SOW. Cyclic AMP via PKA enhances Nav1.8 activity. Alexis Bavencoffe, in collaboration with Dr. Carmen Dessauer in this department, found that blocking cAMP-PKA signaling in nociceptors blocks SCI-induced spontaneous activity (SA) in these neurons, in part through a potential reduction in Nav1.8

Fig.3



activity. As illustrated in Fig.3, SA recorded under current clamp in nociceptors dissociated from SCI animals 4-8 weeks after injury was blocked by either pretreatment (Fig.3A,B) or superfusion after starting patch recording (Fig.3C,D) with several PKA blockers and PKA-AKAP disruptors, including Rp-cAMPS, stHt31, STAD2, and H-89. In combination with biochemical and molecular data from the Dessauer lab, these unexpected results form the basis of a manuscript that is under revision after submission to the *Journal of Neuroscience*.

Task 1d – Investigate behavioral hypersensitivity effects of blocking Nav1.8 activity with single low and high doses of ambroxol.

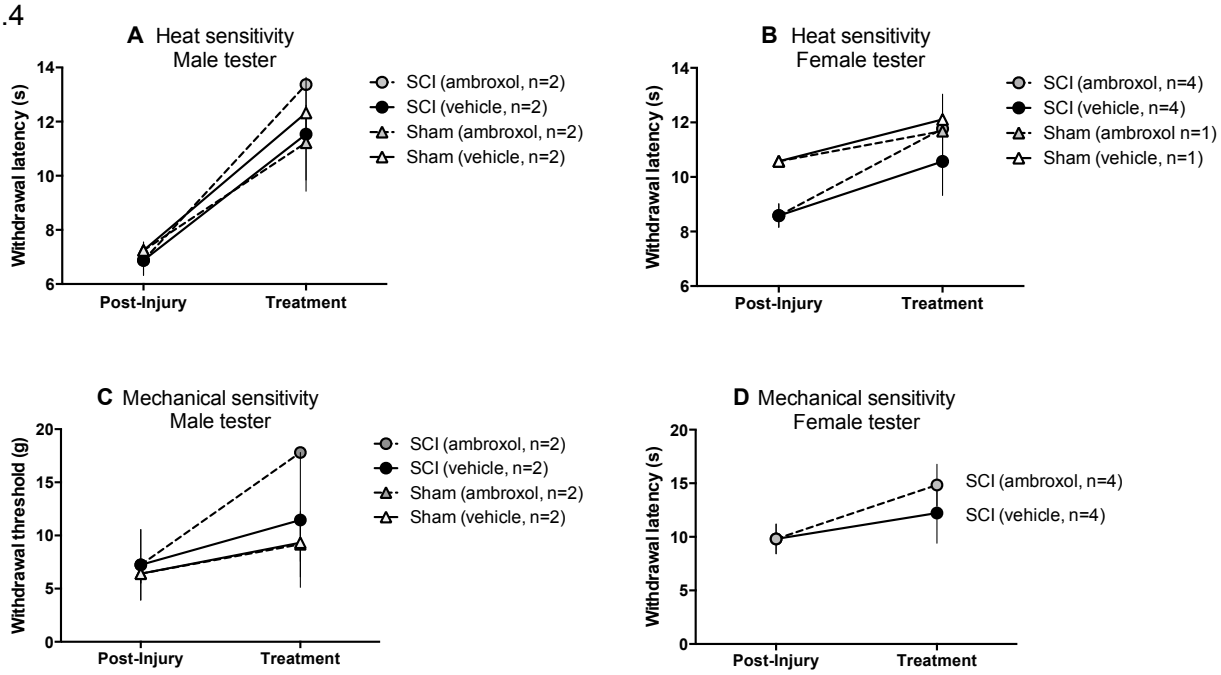
During Year 3 we began to test the hypothesis that the less specific and less costly Nav1.8 blocker, ambroxol, can be used for both brief (Task 1d) and prolonged (Task 1e) attenuation of SCI-induced reflex hypersensitivity. Preliminary studies with single intraperitoneal injections of a high dose of ambroxol 6 weeks after SCI failed to show reliable trends for attenuation of reflex hypersensitivity.

We initially planned to use oral gavage as the delivery procedure. However, our finding that antisense knockdown of Nav1.8 prevented spontaneous pain after SCI, as indicated by blocking of conditioned place preference (CPP), encouraged us to use a delivery route that would be better suited to the CPP procedure. In particular, if ambroxol is to be used as an effective analgesic in the conditioning of place preference, it is necessary that the onset of ambroxol's analgesic effect be rapid so that it can be timed to coincide with placement of the animal in the chamber the animal will learn is a refuge from spontaneous pain. Because oral gavage produces a slow build-up of systemic drug levels, we decided to see if i.p. injection of ambroxol or its vehicle had any analgesic effect or other effects.

We first examined the effect of a single i.p. injection of ambroxol on the sensitivity to heat or mechanical stimulation after SCI and found complicated results. As shown in Fig.4, one complication was that the results depended upon whether testing was performed by a male (Max Odem) or female (Robyn Crook) tester. The male tester found no differences between sham and SCI treatment post-injury in either heat or mechanical sensitivity (Fig. 4A and C). The female tester found an apparent reduction of latency (increased sensitivity) during heat stimulation post-injury compared to the 2 sham animals that were tested (1 in each sham group) (Fig.1B). In addition, all post-injury responses measured by the male tester tended to be sensitized compared to those measured by the female tester. These results extend our tester-gender findings in the A-803467 experiments described in a recent quarterly report (Q9), and are consistent with a recent paper showing that pheromones from young men profoundly influence the injured animals, the presence of male pheromones enhances vigilance, including cutaneous sensitivity,

Injection of ambroxol (100 mg/kg, i.p.) produced larger reversals of hypersensitivity in SCI animals than it did in shams, and larger reversals than produced by vehicle injections (Figs. 4A, B, C, D). This pattern is encouraging for our hypothesis that Nav1.8 antagonism reduces SCI-induced cutaneous

Fig.4

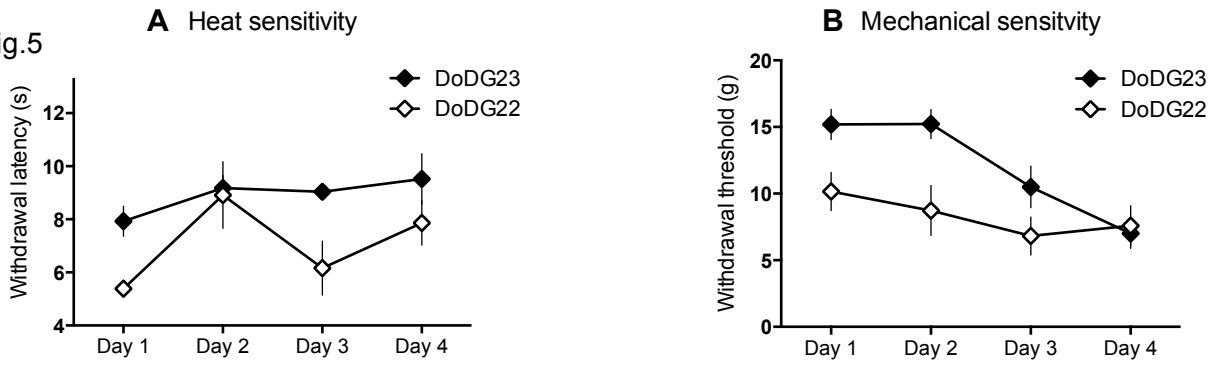


hypersensitivity. However, another complication emerged in these treatment results -- vehicle injection also tended to reverse the cutaneous hypersensitivity. This general effect might represent stress-induced analgesia produced by the needle stick and/or intraperitoneal effects of the vehicle (10% PEG 400 in distilled water). The possibility of stress-induced analgesia is consistent with the larger reversals seen with the male tester, since male pheromone is reported to be analgesic (Sorge et al., 2014).

Given the decreased sensitivity shown in all groups in the treatment test in Fig.4, another potential explanation was that repeated testing by itself could cause the hypersensitivity after injury to habituate. Although this had not been observed in our previous studies, possible habituation might be more prominent with a male tester than the female testers used previously. Therefore, we asked how stable measures of sensitivity to our heat and mechanical stimuli are in naïve (uninjured) animals during

repeated stimulation by a male tester (Alexis Bavencoffe). The results in Fig.4 showed the responses on the second test following one earlier habituating test to the same stimuli, which followed a session of habituation to the testing devices. In the experiment summarized in Fig.5, Dr. Bavencoffe delivered a single up-down mechanical stimulus sequence each morning and a heat stimulus sequence each afternoon for 4 consecutive days, following an initial day of habituation to the testing devices. Two different groups of animals were compared (DoDG22 and DoDG23, each with n=8). Importantly, the differences noted between the tests on Day 2 and Day 3, although substantial in some cases, were opposite to what was observed in the corresponding tests in Fig.4. This argues against habituation (if anything, sensitization was found) as an explanation for the treatment effects in Fig.4. In addition, it was interesting that substantial variability occurred both within each group and between the groups. These complex results add to questions being raised by our findings and by growing numbers of other investigators about how useful these tests of reflex sensitivity are as measures of pain, especially given the more promising results being found with operant measures of pain and anxiety (see below). An

Fig.5



additional approach that we are pursuing is to try different procedures for testing heat and mechanical sensitivity, beginning with standard procedures that Max and Alexis learned in the Ohio State SCI course.

Task 1e – Investigate behavioral hypersensitivity effects of blocking Nav1.8 activity with repeated low and high doses of ambroxol.

Preliminary studies with multiple intraperitoneal injections of a high dose of ambroxol 6 weeks after SCI failed to show any consistent trends for attenuation of reflex hypersensitivity. The preliminary results in Tasks 1d and 1e, as well as Tasks 2d and 2e (Fig.6), plus concerns about potential side effects of the very high doses of ambroxol used both in our studies and all the published claims of analgesic effects from ambroxol, persuaded us that our remaining effort in this project should focus exclusively on the highly specific Nav1.8 antagonists, A-803467 and PF-01247324.

Task 1f – Investigate effects on visceral hypersensitivity of selectively blocking Nav1.8 activity with high doses of A-803467.

We reluctantly abandoned this task. In Year 1 we had performed several of these experiments with animals that received Nav1.8 ASO's via intrathecal catheters, with mixed but somewhat encouraging results. However, we realized that response variability and potentially unnecessary suffering was likely from the cumulative stress of SCI followed by catheterization (which interacts adversely with SCI) followed by numerous tests of forelimb and hindlimb reflexes and finally implantation of recording electrodes before noxious visceromotor testing. Therefore, we decided to limit visceromotor testing to the studies using drug application rather than intrathecal ASO application. Before we could continue these studies a critical member of Dr. Hongzhen Hu's laboratory left in Year 1 and then Dr. Hu himself departed unexpectedly at the end of Year 2. Although Dr. Yang trained in the necessary methods, and Dr. Hu left us necessary equipment for conducting these experiments, Dr Yang did not have time to get to these experiments before she left the project at the end of Year 3. We hope to return to them with a future grant.

Task 2a – Optimize conditions for the use of operant CPP and OC tests to reveal emotional/cognitive features of SCI pain.

During the past year we have continued to work on the most important part of this project - investigating the roles of Nav1.8-dependent nociceptor activity in maintaining the aversive features of pain-related behavior after SCI. In our recent article (Yang et al., 2014) we described the first evidence for ongoing, **spontaneous pain** in a contusive SCI model. We modified a conditioned place preference (**CPP**) procedure that had been used to assess ongoing pain in several other rat pain models. An important difference is that we conditioned place preference to a white chamber paired with retigabine injection. In the same animals, vehicle injection was paired with placement in the black chamber in the 3-chambered box (white-gray-black). Retigabine opens KCNQ K^+ channels, reducing neuronal excitability and, in other models, behavioral hypersensitivity. Importantly, we found that retigabine suppresses SA in small DRG neurons and reverses hyperreflexia after SCI (Yang et al., 2014). Another difference is that we habituated the animals to the black and gray chambers before conditioning, but the animals did not experience the white chamber until it was paired with retigabine injection (increasing the salience of the white chamber and its probable effectiveness as a conditioned context). One day after the 3-day differential conditioning procedure, sham animals preferred the vehicle-paired black chamber, whereas SCI animals showed relative preference for the white, retigabine-paired chamber. Preference for the white chamber in SCI but not sham animals indicates that retigabine is only rewarding when an SCI-induced aversive state is present. Using antisense oligodeoxynucleotides to knock down Nav1.8, we showed that Nav1.8 function is necessary to maintain ongoing pain after SCI. During Year 3 we have confirmed that this CPP procedure is effective and we have taught it to another group (Dr. Annemieke Kavelaars' laboratory at University of Texas MD Anderson Cancer Center), which has confirmed that this new retigabine-based CPP model is effective in another chronic pain model in mice.

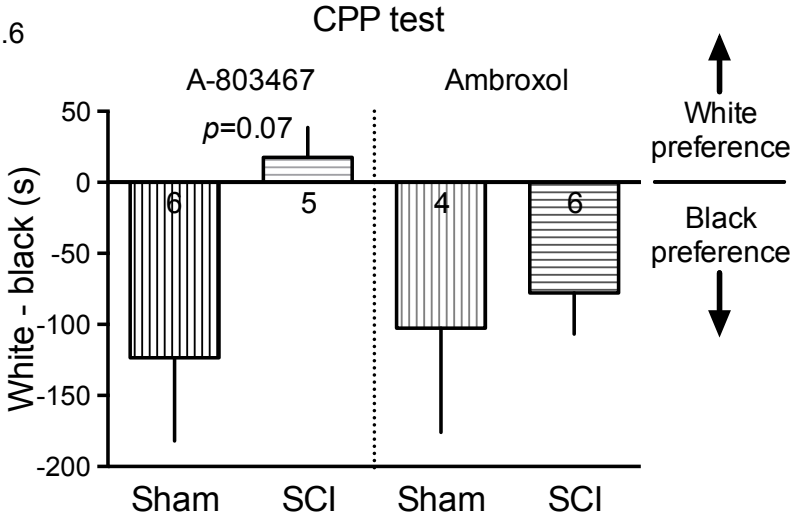
Task 2b – Investigate prolonged effects on spontaneous pain of Nav1.8 knockdown

This task has been completed successfully. Antisense knockdown of Nav1.8 eliminated signs of spontaneous pain 6-8 weeks after SCI, as assessed with the CPP test. The results are described in our recently published paper (Yang et al., 2014).

Task 2c – Investigate brief effects on spontaneous pain from a single application of A-803467.

This task is continuing. Initial studies conducted by Mr. Odem have revealed little or no conditioning of place preference when nociceptor SA is blocked acutely by a single i.p. injection of A-803467 (data not shown). Further experiments using female testers are examining whether the lack of a drug effect represents a failure of brief inhibition of nociceptor SA to provide analgesia, or whether the lack of conditioning is a consequence of complicating stress-induced analgesia produced by the presence of a male tester (Sorge et al., 2014). Indeed, it may be important that the successful conditioning of place preference with retigabine (Yang et al., 2014) was achieved when testing was conducted exclusively by a female (Ms. Julia Hadden). Additional tests by a female research volunteer (Alexa van Brummen) have yielded promising preliminary evidence that place conditioning in SCI rats occurs when injections of A-803467 but not ambroxol are paired with the innately less-preferred white chamber (vehicle injections are paired with the innately preferred black chamber). In these experiments extensive time was spent by each tester in the presence of the rats with frequent handling prior to formal testing so that stressful responses to humans

Fig.6



(especially to human males) habituated. As shown in Fig. 6 (left panel), the preference showed a strong trend to shift from the vehicle-paired black chamber to the A-803467-paired white chamber. This is an important result for several reasons. First, it provides additional evidence for chronic spontaneous pain in rats after contusive SCI. Second, these results were obtained at the same time and, in part, in the same rats and by the same person (Alexa) who failed to find significant heat hypersensitivity after contusive SCI in a much larger sample of rats (Fig.1). This indicates either that spontaneous pain is a more prominent effect of this type of SCI than heat hypersensitivity, or that some aspect of the procedures (e.g., longer time to dissipate stress from drug injections in the CPP procedure) favors the CPP procedure for revealing pain-related behavior. Interestingly, 3 repeated injections of the much less specific Nav1.8 antagonist, ambroxol, failed to show any evidence for relieving spontaneous pain (Fig.6, right panel).

Task 2d, 2e – Investigate brief effects on spontaneous pain from a single or multiple applications of ambroxol.

As shown in Fig.6, our initial attempts to produce CPP with 3 injections of ambroxol across 3 days failed to reveal any evidence that ambroxol relieves spontaneous pain.

Plans for Year 6-month EWOF period

Using improved SCI surgical procedures and reflex testing methods we will complete efforts to demonstrate that blocking Nav1.8 activity *in vivo* with the specific antagonist, A-80346, significantly reduces both chronic hyperreflexia and ongoing, spontaneous pain after SCI. We will also begin to test a newer, highly specific Nav1.8 antagonist on the same behavioral responses to SCI. This drug, PF-01247324, has superior bioavailability and we are making progress in obtaining it as a gift from Pfizer.

4. KEY RESEARCH ACCOMPLISHMENTS

1) Made major progress in improving SCI surgical procedures and reflex testing methods.

2) Further documented the effectiveness of our conditioned place preference procedure for assessing chronic, spontaneous pain.

3) Showed that blockade of a signaling pathway that enhances Nav1.8 activity (the cAMP-PKA pathway) eliminates spontaneous activity in dissociated nociceptors.

4) Made progress in showing that the specific Nav1.8 antagonist, A-803467, reduces reflex hypersensitivity after SCI.

5) Made progress in showing that A-803467 reduces spontaneous pain after SCI.

6) Found that high concentrations of the nonspecific Nav1.8 antagonist, ambroxol, fails to reduce SCI-induced hyperreflexia or spontaneous pain.

7) Added to evidence that rodent responses to pain-testing stimuli are significantly influenced by the gender of the tester, with male testers eliciting complicating stress responses that are much less apparent when testing is performed by females.

5. CONCLUSION

The research accomplishments summarized above provide strong evidence for an important, but previously unappreciated role for ongoing hyperactivity in widespread primary afferent neurons for maintaining spontaneous pain and hypersensitivity of defensive reflexes chronically after contusive SCI. A major implication is that further development of drugs that antagonize Nav1.8 channels could lead to more effective and selective treatments for spontaneous and evoked pain, anxiety, and hyperreflexia after SCI. Furthermore, this model may be revealing a fundamental role for persistent hyperactivity in widespread nociceptors that might also contribute to other conditions involving chronic pain and anxiety, so the potential therapeutic implications may generalize beyond SCI. During the final 6-month EWOF period of this award we will continue to develop this evidence in our SCI model by working hard to complete our demonstration that acute treatment with selective Nav1.8 antagonists rapidly reduces SCI-induced hyperreflexia, spontaneous pain, and anxiety. Because unexpected (but illuminating) results slowed some of the studies, in our remaining time our top priority will be to complete the proposed studies in the statement of work that are most important for documenting and understanding our major discoveries relating to pain- and anxiety-related consequences of ongoing nociceptor hyperactivity after SCI. These discoveries may open up new therapeutic approaches for helping many people, including military personnel, suffering from SCI and related conditions.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS

a. Manuscripts submitted or published

1. Lay press:

The Spin: Spinal Cord Injury BC, 2015, "Rethinking pain" http://sci-bc.ca/stories/spin-magazine/ Spring 2015.

2. Peer-Reviewed Scientific Journals:

Bavencoffe A, Li Y, Wu Z, Yang Q, Herrera J, Kennedy EJ, Walters ET, Dessauer CD. Persistent electrical activity in primary nociceptors after spinal cord injury is maintained by scaffolded adenylyl cyclase and protein kinase A and is associated with altered adenylyl cyclase regulation. *J Neurosci*. (under revision, second round)

3. Invited articles: none

4. Abstracts:

Odem MA, Hadden JK, Crook RJ, Du J, Carlton SM, Yang Q, Walters ET. Inhibition of Nav1.8 channels reduces pain-related behavior after spinal cord injury. Program 537.31. *2014 Abstract Viewer/Itinerary Planner.* Washington DC: Society for Neuroscience, 2014.

Bavencoffe A, Wu Z, Yang Q, Du J, Li Y. Kennedy EJ, Carlton SM, Dessauer CW, Walters ET. AKAP-dependent cAMP-PKA signaling maintains pain-related spontaneous activity in nociceptor somata after spinal cord injury. Program 242.26. *2014 Abstract Viewer/Itinerary Planner.* Washington DC: Society for Neuroscience, 2014.

Bavencoffe A, Yang Q, Bloom O, Walters ET. Does macrophage migration inhibitory factor (MIF) contribute to chronic spontaneous activity in nociceptor somata after spinal cord injury? Program 604.09. *2015 Abstract Viewer/Itinerary Planner.* Chicago IL: Society for Neuroscience, 2015.

b. Presentations:

10/14 Neuroscience Research Seminar Series, Indiana University School of Medicine, Indianapolis, IN

4/15 Gulf Coast Consortium for Translational Pain Research Symposium, Houston, TX

6/15 European Pain School, Siena, Italy

8/15 Texas Pain Research Consortium Conference, University of Texas at Dallas, TX

7. INVENTIONS, PATENTS AND LICENSES: none

8. REPORTABLE OUTCOMES: none other than the papers and presentations described above

9. OTHER ACHIEVEMENTS: none directly related to this award

10. REFERENCES (***** supported in part by this award)**:**

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*Yang, Q, Wu, Z, Hadden, JK, Odem, MA, Zuo, Y, Crook, RJ, Frost, JA, Walters, ET (2014) Persistent pain after spinal cord injury is maintained by primary afferent activity. J Neurosci, 34:10765–10769.

11. APPENDICES

Individuals working on the project

Name	Edgar T. Walters, Ph.D.
Project role	PD/PI
Nearest person month worked	12
Contributions to project	Design, supervision, data analysis, writing, and presentation of results.
Funding support (other than DoD)	National Science Foundation, "Collaborative Research: Comparisons of Fun and Mechanisms of Nociceptive Sensitization in Dissimilar Molluscs". Role - Craig H. Neilsen Foundation, “Contributions of inflammatory mediators in chronic SCI”, Role - collaborator. Mission Connect-TIRR Foundation, "Targeting TRPV1 Channels to Reduce Spontaneous Neuropathic Pain After SCI ". Role - PI.

Name	Qing Yang, M.D.
Project role	Co-PD/PI
Nearest person month worked	12
Contributions to project	Design, electrophysiology, SCI surgery, behavioral tests, animal care, western blot, data analysis, writing.
Funding support (other than DoD)	Mission Connect-TIRR Foundation, "Neuroprotective Effect of Targeting KCNQ/Kv7 Channels in Spinal Cord Injury". Role - PI. American Pain Society, "Novel Target for Preventing & Ameliorating Paclitaxel-Induced Neuropathic Pain". Role - PI.

Name	Alexis Bavencoffe
Project role	Postdoctoral fellow
Nearest person month worked	9
Contributions to project	Electrophysiology, behavioral tests, animal care
Funding support (other than DoD)	

Name	Max Odem
Project role	Research assistant (graduate student)
Nearest person month worked	6
Contributions to project	Design, behavioral tests, animal care, data analysis, SCI surgery
Funding support (other than DoD)	Research assistantship from Graduate School of Biomedical Sciences

Name	Alexa van Brummen
Project role	Research assistant (medical student, summer research)
Nearest person month worked	2
Contributions to project	Behavioral tests, animal care
Funding support (other than DoD)	Endowment to E.T. Walters

Opportunities provided for training and professional development:

The PI continued to offer professional mentoring and career guidance to Dr. Qing Yang (Co-PD/PI and a junior faculty member) and Dr. Alexis Bavencoffe. He also provided extensive guidance and instruction to Mr. Max Odem, a graduate student in the laboratory. Mr. Odem has also benefited from courses and career guidance from the University of Texas at Houston Graduate School of Biomedical Sciences.